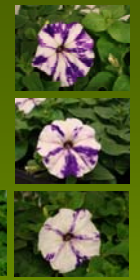
Functional characterization of petunia petal senescence related proteins by virus-induced gene silencing

Shuangyi Bai[1], David Francis[1], Belinda Willard[2], Michael Kinter[2] and Michelle L Jones[1]
[1] Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691
[2] Department of Cell Biology, Cleveland Clinic Foundation, Cleveland, OH 44195



Abstract

The senescence of vegetative and floral tissues can have a detrimental impact on the quality and subsequent value of agricultural and horticultural crops. Pollination, one of the key stimuli of senescence, can trigger and accelerate flower senescence and nutrient recycling. To profile protein expression during flower senescence, we used a proteomic approach to identify components of the senescence program in *Petunia x hybrida* cv Mitchell Diploid petals. Two- dimensional gel electrophoresis (2DE) was used to identify those proteins that were differentially expressed in nonsenescing (unpollinated) and senescing (pollinated) corollas. Proteins that increased in abundance during petal senescence are mainly involved in stress or defense responses, carbohydrate and energy metabolism, and other catabolic processes including proteolysis, nuclei acid, cell wall and lipid degradation. Since virus-induced gene silencing (VIGS) is a high throughput transient approach to analyze gene function, we are employing VIGS to further investigate the function of the senescence up regulated proteins. A fragment of the petunia chalcone synthase gene (*CHS*) and a fragment of the target gene will be ligated in tandem into the TRV2 vector. Both Agroinfiltration and Agrodrench can induce high efficiency gene silencing in petunia flowers. Currently we are studying the function of two proteins. One is a nuclease that is up regulated in senescing petals. Its molecular weight is very close to that of PhNUC1 whose biochemical characterization has been previously studied by the Jones lab. Another protein is beta-D-xylosidase, which is likely to be related to cell wall disassembly and loosening. This is a highly abundant protein, whose full-length, N-terminal and C-terminal truncated forms were all up regulated during petal senescence. It indicated that this protein might be activated after post-translational modification or processing during petal senescence.

Introduction

Senescence represents the last stage of flower development leading to death. The programmed death of flower petals is an active process that is executed via a defined genetic program (Jones, 2004). Pollination accelerates petal senescence and allows the plant to break down macromolecules and organelles and remobilize nutrients to developing tissues (Langston et al., 2005; Jones et al., 2005). Petal senescence is accompanied by changes in the activity of specific enzymes and the abundance of certain proteins (Jones, 2004). The Jones lab has performed a proteomic approach, utilizing 2-dimensional gel electrophoresis (2-DE) and mass spectroscopy to identify proteins that were differentially expressed in senescing and nonsenescing petunia petals. Many of the proteins that we found are involved in the degradation of carbohydrates, proteins, lipids and nucleic acids. We therefore hypothesize that silencing gene expression of the senescence up regulated proteins will delay petal senescence. We are employing VIGS to further investigate the function of up regulated proteins during flower senescence.

Results

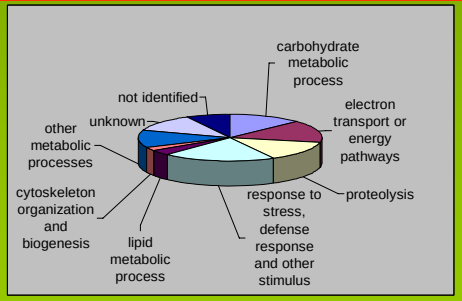


Figure 4. Biological process classification of up regulated proteins during petal senescence based on GO annotations. The majority of proteins are involved in response to stress, defense and other stimulus and macromolecule metabolic processes.

Figure 8. A candidate gene fragment (300-400 bp) will be ligated with a reporter gene *CHS* fragment (~200 bp) in tandem into TRV2 vector (Chen et al., 2004). Four weeks after Agro-infiltration or Agro-drench into seedlings of *Petunia X hybrida* cv Primetime Blue (data not shown) and Fantasy Blue, the purple flowers will show white sectors or whole white petals due to *CHS* gene silencing. The plants from left to right are five weeks after infiltrated with Buffer, TRV2- *PhCHS*, TRV2- *PhCHS -PhLEX2* and TRV2- *PhCHS -PhNUC*. The target gene knockdown levels and senescence effects are being investigated.

Discussion

Materials and Methods

Figure 1. Pollination accelerates petunia flower senescence. Petal wilting is apparent at 48 h after pollination. U, unpollinated; P, pollinated.

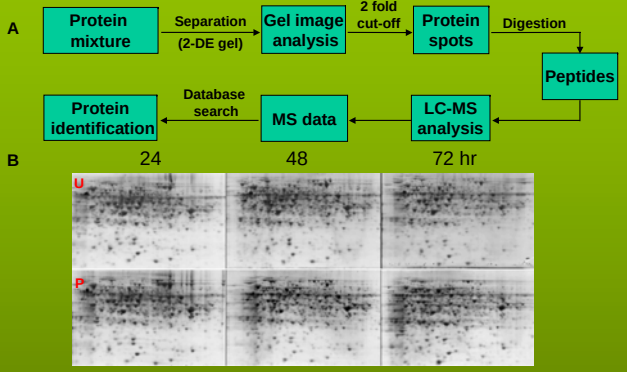


Figure 2. A. Flow chart showing how we identified the senescence-related proteins. **B.** Expression profiling of petal proteins during pollination-induced petunia flower senescence using 2-DE. Representative gels from the 3 replicates comparing protein profiles of corollas from unpollinated (U) flowers and pollinated (P) flowers at 24, 48, and 72 h.

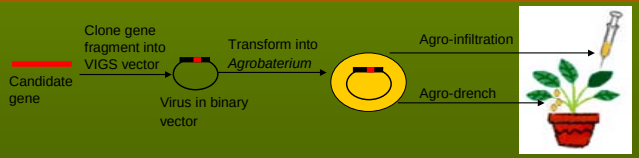


Figure 3. Procedures of virus induced gene silencing in plants (Burch-Smith et al., 2004). The candidate gene fragment (100-500 bp) will be cloned into tobacco rattle virus subgenome 2 (TRV2) driven by CaMV 35S promoter within a binary vector. Both TRV1 and TRV2 constructs will be transformed into *Agrobaterium* and leaves will be infiltrated or roots drenched with 1:1 ratio. The symptoms may show on the plants two weeks after inoculation due to the target gene silencing.

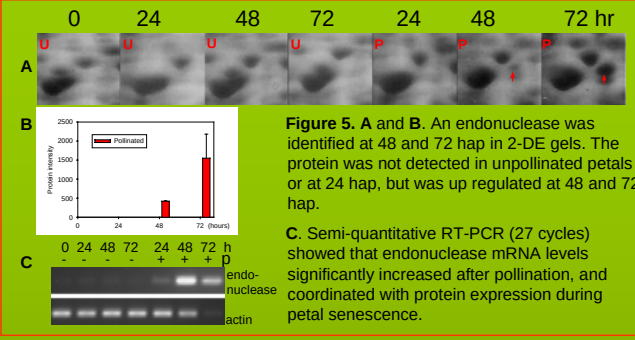


Figure 5. A and **B**. An endonuclease was identified at 48 and 72 hap in 2-DE gels. The protein was not detected in unpollinated petals or at 24 hap, but was up regulated at 48 and 72 hap.

C. Semi-quantitative RT-PCR (27 cycles) showed that endonuclease mRNA levels significantly increased after pollination, and coordinated with protein expression during petal senescence.

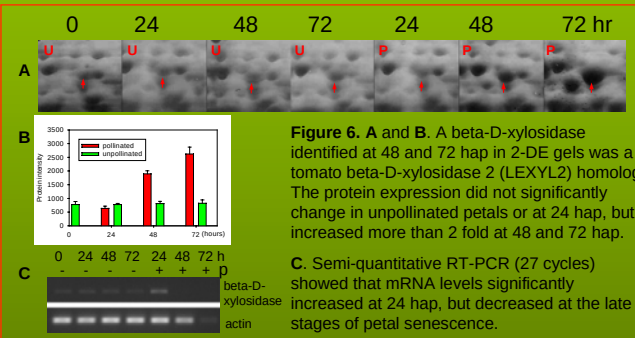


Figure 6. A and **B**. A beta-D-xylosidase identified at 48 and 72 hap in 2-DE gels was a tomato beta-D-xylosidase 2 (LEXYL2) homolog. The protein expression did not significantly change in unpollinated petals or at 24 hap, but increased more than 2 fold at 48 and 72 hap.

C. Semi-quantitative RT-PCR (27 cycles) showed that mRNA levels significantly increased at 24 hap, but decreased at the late stages of petal senescence.

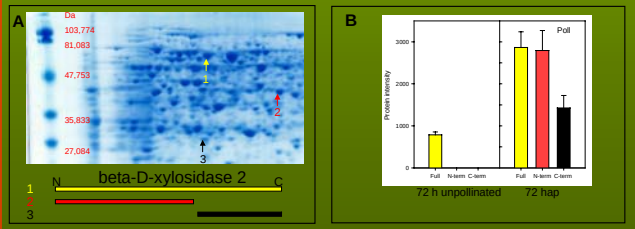


Figure 7. A. Three protein spots were identified from 72 hap as full-length, N-terminal and C-terminal truncated forms of beta-D-xylosidase 2.

B. Beta-D-xylosidase 2 full-length protein expression increased significantly compared with 72 hour unpollinated petals. Both N-terminal and C-terminal forms of beta-D-xylosidase 2 were only detected at 72 hap.

The majority of the up regulated proteins identified by 2-DE during petunia petal senescence were involved in catabolic processes and stress responses. We chose an endonuclease and a beta-D-xylosidase 2 protein for further analysis. Endonucleases are involved in nucleic acid catabolism during senescence, which allows the plant to remobilize nutrients, primary phosphorus, from senescing to developing tissues. The endonuclease identified in 2-DE gels has a molecular weight of 43 kDa, and it may be the protein responsible for the senescence-specific nuclease (PhNUC1) activity we have previously characterized in petunia corollas (Langston et al., 2005). Gene expression of the endonuclease corresponds with protein expression patterns.

Beta-D-xylosidase 2 is an enzyme involved in cell wall polysaccharide disassembly or modification and may function in cell wall degradation during senescence. The up regulation of the N- and C-terminally truncated forms of the beta-D-xylosidase 2 protein during senescence suggest that it may be targeted for degradation late in the senescence program or that post translational cleavage may be required to activate the enzyme during senescence. To date, the specific function of beta-D-xylosidase 2 enzymes has not been characterized.

We have successfully knocked down *CHS* gene expression by VIGS and both Agroinfiltration and Agrodrench can induce high efficiency gene silencing in petunia flowers. Functional analysis of the endonuclease and beta-D-xylosidase 2 proteins using VIGS is currently underway. We hypothesize that silencing these proteins will delay petal senescence.

Literature cited

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